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(54) Title: OLIGODEOXYNUCLEOTIDE AND ITS USE TO INDUCE AN IMMUNE RESPONSE

(57) Abstract: The present invention provides a substantially pure or isolated oligodeoxynucleotide (ODN) of at least about 10 nucleotides comprising different CpG sequences, as well as an oligodeoxynucleotide delivery complex and a pharmacological composition comprising an ODN or ODNs, and a method of inducing an immune response by administering such an ODN or ODNs to a host.

OLIGODEOXYNUCLEOTIDE AND ITS USE TO INDUCE AN IMMUNE RESPONSE

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

This patent application claims the benefit of U.S. provisional patent application 60/176,115, filed January 14, 2000.

TECHNICAL FIELD OF THE INVENTION

The present invention pertains generally to induction of an immune response using different CpG sequences.

BACKGROUND OF THE INVENTION

DNA is a complex macromolecule whose immunological activities are influenced by its base composition and base modification, as well as helical orientation. Certain unusual DNA structures (e.g., Z-DNA) can induce significant antibody responses when administered to normal mice. In addition, bacterial DNA, as well as certain synthetic unmethylated CpG sequences can induce proliferation and immunoglobulin (Ig) production by murine B cells. Unmethylated CpG dinucleotides are more frequent in the genomes of bacteria and viruses than vertebrates, and recent studies suggest that immune recognition of these motifs may contribute to the host's innate immune response. D.M. Klinman et al., *CpG Motifs Present in Bacterial DNA Rapidly Induce Lymphocytes to Secrete Interleukin 6, Interleukin 12, and Interferon γ*, 93 Proc. Natl. Acad. Sci. USA 2879 (1996); A.-K. Yi et al., *Rapid Immune Activation by CpG Motifs in Bacterial DNA*, 157 J. Immun. 5394 (1996); Hua Liang et al., *Activation of Human B Cells by Phosphorothioate Oligodeoxynucleotides*, 98 J. Clin. Invest. 1119 (1996); A.M. Krieg et al., *CpG Motifs in Bacterial DNA Trigger Direct B-Cell Activation*, 374 Nature 546 (1995).

In mice, CpG DNA induces proliferation in almost all (>95%) of B cells and increases Ig secretion. This B cell activation by CpG DNA is T-cell independent and antigen non-specific. In addition to its direct effects on B cells, CpG DNA also directly activates monocytes, macrophages, and dendritic cells to secrete a variety of

cytokines. These cytokines stimulate natural killer (NK) cells to secrete γ-interferon (IFN-γ) and have increased lytic activity. Examples of which can be found in International Patent Applications WO 95/26204, WO 96/02555, WO 98/11211, WO 98/18810, WO 98/37919, WO 98/40100, WO 98/52581; U.S. Patent Application Nos. 08/738,652; and U.S. Patent No. 5,663,153.

Although bacterial DNA and certain CpG sequences can induce responses from human cells (Z.K. Ballas et al., *Induction of NK Activity in Murine and Human Cells by CpG Motifs in Oligodeoxynucleotides and Bacterial DNA*, 157 J. Immunol. 1840 (1996)). individual subjects show considerable heterogeneity in their response to different CpG sequences. Indeed, CpG sequences that strongly stimulate cells from some subjects are virtually inactive on cells from other subjects. These different responses make it difficult, it not impossible, to induce a therapeutic immune response in all members of a diverse population using a single CpG sequence, even if such a sequence is expressed repetitively in a given oligonucleotide.

In view of the above, there exists a need to identify different CpG sequences that together are capable of optimally inducing an immune response in cells from all members of a target population. In addition, there is a need for methods utilizing these CpG sequences in the treatment of diseases. The present invention provides such CpG sequences and methods of use. These and other advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a substantially pure or isolated oligodeoxynucleotide (ODN) of at least about 10 nucleotides comprising multiple CpG sequences, wherein at least one of the CpG sequences is different from another of the multiple CpG sequences. The present invention also provides an ODN delivery complex and a pharmacological composition comprising an ODN or ODNs, as well as a method of inducing an immune response by administering an ODN or ODNs to a host.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery that different CpG sequences, which are formulated either as multiple individual oligodeoxynucleotides (ODNs) each comprising a single CpG motif, or a complex ODN comprising multiple CpG sequences, induces an enhanced immune response in a broad population.

Oligodeoxynucleotide

The present invention provides novel ODNs. These ODNs have at least about 10 nucleotides and comprise multiple (i.e., 2 or more) CpG sequences, wherein at least one of the multiple CpG sequences is different from another of the multiple CpG sequences. A "CpG sequence" or "CpG motif" refers to a nucleic acid sequence having a cytosine followed by a guanine linked by a phosphate bond in which the cytosine is unmethylated.

Preferably, the ODNs of the present invention comprise multiple different CpG sequences with at least one of the multiple different CpG sequences represented by the either formula 5' $N_1N_2N_3$T-CpG-W$N_4N_5N_6$ 3', wherein W is A or T, and N_1, N_2, N_3, N_4, N_5, and N_6 are any nucleotides, or the formula 5' RY-CpG-RY 3', wherein R is A or G and Y is C or T. Alternatively, two, three, or more of the multiple different sequences can be represented by either the formula 5' $N_1N_2N_3$T-CpG-W$N_4N_5N_6$ 3', wherein W is A or T, and N_1, N_2, N_3, N_4, N_5, and N_6 are any nucleotides, or the formula 5' RY-CpG-RY 3', wherein R is A or G and Y is C or T. Thus, at least one of the different CpG sequences can be selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:112. Alternatively, two, three, or more of the different CpG sequences can be selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:112.

Preferably, the ODN of the present invention is substantially pure or isolated. "Substantially pure" refers to an ODN that is substantially free of other materials, particularly other nucleic acids, proteins, lipids, carbohydrates, and other materials with which it may be naturally associated, while "isolated" refers to an ODN that is removed from its natural environment or state. The ODN of the present invention

can consist of any suitable number of nucleotides. For example. the ODN can consist of about 100 nucleotides or less (e.g., about 10-75 nucleotides) or about 50 nucleotides or less (e.g., about 10-40 nucleotides).

Preferably, the ODNs inducing a humoral immune response, e.g., those containing at least one sequence represented by the formula 5' $N_1N_2N_3$T-CpG-$WN_4N_5N_6$ 3', contain a phosphate backbone modification, and more preferably, the phosphate backbone modification is a phosphorothioate backbone modification (i.e., one of the non-bridging oxygens is replaced with sulfur, as set forth in International Patent Application WO 95/26204). For the ODNs inducing a cell-mediated immune response and containing a phosphodiester backbone, e.g., those containing at least one sequence represented by the formula 5' RY-CpG-RY 3', the ODN preferably has been modified to prevent degradation.

Any suitable modification can be used in the present invention to render the ODN resistant to *in vivo* degradation resulting from, e.g., exo or endonuclease digestion. Preferably, the modification includes a phosphorothioate modification. The phosphorothioate modifications can occur at either termini, e.g., the last two or three 5' and/or 3' nucleotides can be liked with phosphorothioate bonds. The ODN also can be modified to contain a secondary structure (e.g., stem loop structure) such that it is resistant to degradation. Another modification that renders the ODN less susceptible to degradation is the inclusion of nontraditional bases such as inosine and quesine, as well as acetyl-, thio- and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine. Other modified nucleotides include nonionic DNA analogs, such as alkyl or aryl phosphonates (i.e., the charged phosphonate oxygen is replaced with an alkyl or aryl group, as set forth in U.S. Patent No. 4,469,863), phosphodiesters and alkylphosphotriesters (i.e., the charged oxygen moiety is alkylated, as set forth in U.S. Patent No. 5,023,243 and European Patent No. 0 092 574). ODNs containing a diol, such as tetraethyleneglycol or hexaethyleneglycol, at either or both termini, have also been shown to be more resistant to degradation.

Oligodeoxynucleotide Delivery Complex

The present inventive ODN delivery complex can comprise multiple (i.e., more than one) substantially pure or isolated ODNs of at least about 10 nucleotides comprising a CpG sequence and a targeting means, wherein at least one of the CpG sequences is different from another of the multiple CpG sequences. Therefore, the present inventive ODN delivery complex can comprise multiple ODNs comprising a single CpG sequence with at least one of these multiple ODNs comprising a CpG sequence that is different from the CpG sequences comprised by another ODN within the complex.

Additionally, the present inventive ODN delivery complex can comprise a single ODN or multiple ODNs comprising multiple different CpG sequences and a targeting means. Therefore, the present inventive ODN delivery complex can comprise either a single ODN, or multiple (i.e., more than one) ODNs.

Any suitable targeting means (i.e., a molecule that results in higher affinity binding to a target cell) can be used within the context of the present invention. Suitable targeting means are well known in the art. The ODN delivery complex can be associated with (e.g., ionically or covalently bound to, or encapsulated within) the targeting means by a variety of coupling or cross-linking agents, e.g., protein A, carbodiamide, and N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP). Examples of ODN delivery complexes include ODNs associated with a sterol (e.g., cholesterol), a lipid (e.g., a cationic lipid, virosome or liposome), and a target cell specific binding agent (e.g., a ligand recognized by target cell specific receptor). Preferred complexes must be sufficiently stable *in vivo* to prevent significant uncoupling prior to internalization by the target cell; however, these complexes can be cleavable under appropriate circumstances such that the ODN can be released in a functional form.

Pharmacological Composition

The present inventive composition can comprise multiple substantially pure or isolated ODNs of at least about 10 nucleotides comprising a CpG sequence and a pharmacologically acceptable carrier, wherein at least one of the CpG sequences is different from another of the multiple CpG sequences. Therefore, the present

inventive pharmacological composition can comprise multiple ODNs comprising a single CpG sequence and at least one of these multiple ODNs comprises a CpG sequence that is different from the CpG sequences comprised by another ODN within the composition.

Additionally, the present inventive pharmaceutical composition can comprise a single ODN or multiple ODNs comprising multiple different CpG sequences and a pharmacologically acceptable carrier. Therefore, the present inventive pharmaceutical composition can comprise either a single ODN, or multiple (i.e., more than one) ODNs.

Pharmacologically acceptable carriers (e.g., physiologically or pharmaceutically acceptable carriers) are well known in the art. The present inventive pharmacological composition facilitates the use of the one or more present inventive ODNs, both *in vivo* and *ex vivo*. Such a composition can be suitable for delivery of the active ingredient to any suitable host, such as a patient for medical application, and can be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmacological compositions for use in accordance with the present invention can be formulated in a conventional manner using one or more pharmacologically (e.g., physiologically or pharmaceutically) acceptable carriers comprising excipients, as well as optional auxiliaries that facilitate processing of the active compounds into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Thus, for injection, the active ingredient can be formulated in aqueous solutions, preferably in physiologically compatible buffers. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. For oral administration, the active ingredient can be combined with carriers suitable for inclusion into tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like. For administration by inhalation, the active ingredient is conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the

use of a suitable propellant. The active ingredient can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Such compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Other pharmacological excipients are known in the art.

Method of Inducing an Immune Response

The present inventive method of inducing an immune response can comprise administering to a host multiple substantially pure or isolated ODNs of at least about 10 nucleotides comprising a CpG sequence in order to induce an immune response in the host, wherein at least one of the CpG sequences is different from another of the multiple CpG sequences. Therefore, the present inventive method can comprise administering to a host multiple ODNs comprising a single CpG sequence and at least one of these multiple ODNs comprises a CpG sequence that is different from the CpG sequences comprised by another ODN administered to a host.

Additionally, the present inventive method of inducing an immune response can comprise administering to a host a single ODN or multiple ODNs comprising multiple different CpG sequences in order to induce an immune response in the host. Therefore, the present inventive method of inducing an immune response can comprise administering either a single ODN or multiple (i.e., more than one) ODNs to a host in order to induce an immune response in the host.

Administration can be by any suitable method. For example, the ODN or ODNs can be administered *in vivo* or *ex vivo*. Preferably, the ODN or ODNs are administered *in vivo* to a mammal, particularly a human. Optionally, the ODN or ODNs can be contained within or conjugated with a larger nucleic acid molecule, protein, hydrocarbon or lipid. Once this molecule is administered, the CpG sequences must be exposed on the surface to induce an immune response. Examples of suitable nucleic acid molecules include fusion or chimeric nucleic acids, proteins, hydrocarbons and lipids. The ODN or ODNs can also be co-administered with another nucleic acid, protein, hydrocarbon, or lipid. Co-administration can be such that the ODN or ODNs are administered before, at substantially the same time as, or

after the other nucleic acid, protein, hydrocarbon, or lipid. Preferably, the ODN or ODNs are administered at substantially the same time as the other nucleic acid, protein, hydrocarbon, or lipid.

After administration of the ODN or ODNs, while not intending to be bound by any particular theory, it is thought that the ODN initially acts on antigen presenting cells (e.g., macrophages and dendritic cells). These cells then release cytokines, which activate natural killer (NK) cells. Either a cell-mediated or humoral immune response then occurs in the host.

The cell-mediated or local immune response is produced by T cells, which are able to detect the presence of invading pathogens through a recognition system referred to as the T cell antigen receptor. Upon detection of an antigen, T cells direct the release of multiple T cell cytokines, including IL-2, IL-3, IFN-γ, TNF-β, GM-CSF and high levels of TNF-α, and chemokines MIP-1α, MIP-1β, and RANTES. IL-2 is a T cell growth factor that promotes the production of additional T cells sensitive to the particular antigen. This production constitutes a clone of the T cells. The sensitized T cells attach to cells containing the antigen. T cells carry out a variety of regulatory and defense functions and play a central role in immunologic responses. When stimulated to produce a cell-mediated immune response, some T cells respond by acting as killer cells, killing the host's own cells when these cells are infected or cancerous and therefore recognized as foreign. Some T cells respond by stimulating B cells, while other T cells respond by suppressing immune response. If a cell-mediated immune response is induced, preferably, non-B cells are activated, more preferably, cytokines are produced, and most preferably, IFN-γ is produced.

The humoral or systemic immune response depends on the ability of the B cells to recognize specific antigens. The mechanism by which B cells recognize antigens is through specific receptors on the surface of the B cells. When an antigen attaches to the receptor site of a B cell, the B cell is stimulated to divide. The daughter cells become plasma cells that manufacture antibodies complementary to the attached antigen. Each plasma cell produces thousands of antibody molecules per minute, which are released into the bloodstream. Many B cells appear to be regulated by the helper T cells and suppressor T cells and produce various cytokines, e.g., IL-3,

IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, GM-CSF and low levels of TNF-α. Helper T cells stimulate B cells to produce antibodies against antigens, while suppressor T cells inhibit antibody production. Some B cells, however, are T cell independent and require no stimulation by the T cells. If a humoral immune response is induced, preferably, B cells are activated, more preferably, IL-6 is produced, and most preferably, antibodies are produced.

In addition, induction of one type of immune response may allow for immune regulation because up regulation of one type of immune response may down regulate the other type of immune response. This immune regulation allows for customizing or tailoring of the type of immune response when administering an ODN.

The present inventive method of inducing an immune response can be used to treat, prevent, or ameliorate any suitable allergic reaction. Optionally, the present inventive method can be used in combination with any suitable anti-allergenic agent. An allergy, in the context of the present invention, refers to an acquired hypersensitivity to a substance (i.e., an allergen). Allergic conditions include eczema, allergic rhinitis or coryza, hay fever, bronchial asthma, uticaria (hives), food allergies, and other atopic conditions. The list of allergens is extensive and includes pollens, insect venoms, animal dander, dust, fungal spores, and drugs (e.g., penicillin). Examples of natural, animal, and plant allergens can be found in International Patent Application WO 98/18810. Preferably, the present inventive method is used to treat allergic asthma. Suitable anti-allergenic agents include those substances given in treatment of the various allergic conditions described above, examples of which can be found in the Physicians' Desk Reference (1998).

The present inventive method of inducing an immune response can be used to treat any suitable cancer. Optionally, the present inventive method can be used in combination with any suitable anti-cancer agent. Suitable cancers include cancers of the brain, lung (e.g., small cell and non-small cell), ovary, breast, prostate, and colon, as well as carcinomas and sarcomas. Preferably, the present inventive method is used to treat a solid tumor cancer. Suitable anti-cancer agents include those substances given in treatment of the various conditions described above, examples of which can be found in the Physicians' Desk Reference (1998).

The present inventive method of inducing an immune response can be used to improve the efficacy of any suitable vaccine. Suitable vaccines include those directed against Hepatitis A, B, and C, examples of which can be found in the Physicians' Desk Reference (1998), and DNA vaccines directed against HIV and malaria. *See generally* D. Klinman et al., *CpG Motifs as Immune Adjuvants*, 17 Vaccine 19 (1999); M.J. McCluskie and H.L. Davis, *CpG DNA is a Potent Enhancer of Systemic & Mucosal Immune Response Against Hepatitis B Surface Antigen with Intra-Nasal Administration to Mice*, 161 J. Immun. 4463 (1998).

The present inventive method of inducing an immune response can be used to treat, prevent, or ameliorate any suitable disease associated with the immune system. Preferred diseases associated with the immune system are autoimmune disorders and immune system deficiencies, e.g., lupus erythematosus, and autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. Immune system deficiencies include those diseases or disorders in which the immune system is not functioning at normal capacity, or in which it would be useful to boost the immune system response.

The present inventive method of inducing an immune response can be used with any suitable antisense therapy. Optionally, the present inventive method can be used in combination any suitable antisense agent. Suitable antisense agents are those that bind either with DNA or RNA and block their function by inhibiting expression of the sequence to which the antisense agents are bound. *See generally* H. Lonnberg et al., *Towards Genomic Drug Therapy with Antisense Oligonucleotides*, 28 Ann. Med. 511 (1996); A. Alama et al., *Antisense Oligonucleotides as Therapeutic Agents*, 36 Pharmacol. Res. 171 (1997); K.J. Scanlon et al., *Oligonucleotide-Mediated Modulation of Mammalian Gene Expression*, 9 FASEB J. 1288 (1995); R. Oberbauer, *Not Non-Sense but Antisense -- Applications of Antisense Oligonucleotides in Different Fields of Medicine*, 109 Wien Klin Wochenschr 40 (1997).

The present inventive method of inducing an immune response can be used to treat, prevent, or ameliorate any suitable infection. Optionally, the present inventive method can be used in combination with any suitable anti-infectious agent. Examples of infections include francisella, schistosomiasis, tuberculosis, AIDS,

malaria, and leishmania. Examples of suitable infectious viruses, bacteria, fungi, and other organisms (e.g., protists) can be found in International Patent Application WO 98/18810. Suitable anti-infectious agents include those substances given in treatment of the various conditions described elsewhere, examples of which can be found in the Physicians' Desk Reference (1998).

The present inventive method of inducing an immune response can be used to treat, prevent, or ameliorate the symptoms resulting from exposure to a bio-warfare agent. Suitable bio-warfare agents include those naturally occurring biological agents that have been specifically modified in the laboratory. Often, modification of these agents has altered them such that there is no known treatment. Examples include Ebola, Anthrax, and Listeria. In the course of ameliorating the symptoms after exposure, use of the present inventive ODNs may not cure the patient, but rather can extend the patient's life sufficiently such that some other treatment can then be applied.

The present invention is further described in the following examples. These examples are intended only to illustrate the invention and are not intended to limit the scope of the invention in any way.

EXAMPLES

Example 1

The following example demonstrates the varied immune response induced *in vitro* in different donors after administration of an ODN comprising a single CpG sequence. Induction of an immune response was measured by production of the cytokines IL-6 and IFN-γ, and cell proliferation in human peripheral blood mononuclear cells (PBMC) isolated from individual donors.

PBMC were isolated, as described elsewhere (Z.K. Ballas et al., 85 J. Allergy Clin. Immunol. 453 (1990); Z.K. Ballas and W. Rasmussen, 45 J. Immunol. 1039 (1990); Z.K. Ballas and W. Rasmussen, 150 J. Immunol. 17 (1993)). ODNs were synthesized on a DNA synthesizer (Applied Biosystems Inc., Foster City, CA), as described elsewhere (Beacage and Caruthers, *Deoxynucleoside Phosphoramidites – A New Class of Key Intermediates for Deoxypolynucleotide Synthesis*, 22 Tetrahedron

Letters 1859 (1981)). In some ODNs, the normal DNA backbone phosphodiesterase linkages were replaced with phosphorothioate linkages, as described elsewhere (Agrawal et al., 94 Proc. Natl. Acad. Sci. USA 2620 (1997); Agrawal 14 TIB TECH 376 (1996)). To reduce degradation of the ODNs, those that did not have an entire phosphorothioate backbone contained phosphorothioate linkages at the 5' and 3' ends. Cells from the different donors were incubated for approximately 72 hrs with the various ODNs. IL-6 and IFN-γ levels were determined by ELISA using anti-IL-6 and anti-IFN-γ antibodies, as described elsewhere (Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 1989). Cell proliferation was determined by [^{3}H] thymidine incorporation, as described elsewhere (Liang et al., 98 J. Clin. Invest. at 1121). IL-6 levels are set forth in Table 1: Induction of an Immune Response (IL-6), IFN-γ levels are set forth in Table 5: Induction of an Immune Response (IFN-γ), and cell proliferation is set forth in Table 6: Induction of an Immune Response (Cell Proliferation).

TABLE 1: Induction of an Immune Response (IL-6)

	3	4	7	8	15	16	17	18	19	23	24	25	26	27
SEQ ID NO:1	35	53	19	12	9	2	8	6	33	15	5	40	13	3
SEQ ID NO:18	2	3	25	65	--	--	--	--	--	8	3	3	1	1
SEQ ID NO:20	50	45	9	43	5	9	20	20	20	--	12	57	13	3
SEQ ID NO:98	18	42	9	41	60	80	25	11	22	17	6	12	2	1
SEQ ID NO:105	--	--	--	--	--	--	--	--	--	13	7	16	3	1

TABLE 2: Induction of an Immune Response (IFN-γ)

	3	4	5	9	13	14	15	17
SEQ ID NO:40	154	64	6	2	5	11	15	3
SEQ ID NO:42	92	56	419	28	8	4	4	8
SEQ ID NO:43	13	3	269	93	15	6	5	8
SEQ ID NO:100	22	2	16	2	9	9	9	6
SEQ ID NO:101	3	2	15	25	0	16	5	5
SEQ ID NO:102	--	--	0	1	60	250	6	3
SEQ ID NO:103	--	--	4	1	3	2	8	5
SEQ ID NO:104	--	--	4	1	2	1	3	4

TABLE 3: Induction of an Immune Response (Cell Proliferation)

	1	2	3	4	20	21	22
SEQ ID NO:1	22	36	33	9	18	15	14
SEQ ID NO:9	5	17	16	11	18	18	13
SEQ ID NO:10	9	10	20	25	14	23	12
SEQ ID NO:12	6	8	10	19	4	7	6
SEQ ID NO:15	9	47	11	13	14	17	12
SEQ ID NO:31	2	15	10	16	6	8	7
SEQ ID NO:45	3	7	16	15	10	16	10
SEQ ID NO:50	3	6	10	9	6	6	5
SEQ ID NO:54	4	5	10	9	5	5	4

The foregoing data demonstrates induction of an immune response to an ODN comprising various sequences in human PBMC isolated from individual donors. Specifically, these data demonstrate that a single sequence induces a varied immune response in different donors, as show, e.g., in Table 4, an ODN comprising SEQ ID NO:98 induced IL-6 levels ranging from 2 to 80, in Table 5, an ODN comprising SEQ ID NO:42 induced IFN-γ levels ranging from 4 to 419, and in Table 6, an ODN comprising SEQ ID NO:15 induced cell proliferation ranging from 9 to 47.

Example 2

The following example demonstrates the varied immune responses induced *in vitro* in different donors after administration of an ODN comprising a single CpG sequence. Induction of an immune response was measured by production of the cytokines IL-6 and IFN-γ in human PBMC isolated from individual donors as compared to unstimulated PBMC from the same donor.

Human PBMC were isolated, as described in Example 1. ODNs were synthesized on a DNA synthesizer (Applied Biosystems Inc., Foster City, CA), as described in Example 1. In some ODNs, the normal DNA backbone phosphodiesterase linkages were replaced with phosphorothioate linkages, as described in Example 1. To reduce degradation of the ODNs, those that did not have an entire phosphorothioate backbone contained phosphorothioate linkages at the 5' and 3' ends. Cells were incubated for approximately 72 hrs with the various ODNs.

IL-6 and IFN-γ levels were determined by ELISA using anti-IL-6 and anti-IFN-γ antibodies, as described in Example 1. The percentage of donors induced by the various ODNs at least 3-fold for IL-6 and 5-fold for IFN-γ are set forth for IL-6 levels in Table 4: Percent Induction of an Immune Response (IL-6) and for IFN-γ levels in Table 5: Induction of an Immune Response (IFN-γ). Also, a profile was created for some of the donors in which greater than a 10-fold increase is represented by "++", greater than a 3-fold increase for IL-6 and greater than a 5-fold increase for IFN-γ is represented by "+"and any levels lower than this are represented by "--". These profiles are set forth for IL-6 in Table 6: Heterogeneity in Induction of an Immune Response (IL-6) and for IFN-γ in Table 7: Heterogeneity in Induction of an Immune Response (IFN-γ).

TABLE 4: Percent Induction of an Immune Response (IL-6)

	Percent Induction	Number of Donors
SEQ ID NO:1	69%	26
SEQ ID NO:109	67%	9
SEQ ID NO:20	65%	34
SEQ ID NO:7	49%	39
SEQ ID NO:108	47%	38
SEQ ID NO:98	44%	39
SEQ ID NO:110	30%	10
SEQ ID NO:111	26%	19
SEQ ID NO:112	7%	29

TABLE 5: Percent Induction of an Immune Response (IFN-γ)

	Percent Induction	Number of Donors
SEQ ID NO:43	93%	42
SEQ ID NO:42	91%	23
SEQ ID NO:106	87%	23
SEQ ID NO:32	82%	17
SEQ ID NO:40	79%	19
SEQ ID NO:103	58%	12
SEQ ID NO:102	57%	28
SEQ ID NO:107	10%	31

	Percent Induction	Number of Donors
SEQ ID NO:68	11%	19

TABLE 6: Heterogeneity in Induction of an Immune Response (IL-6)

	1	2	3	4	5	6	7	8
SEQ ID NO:20	--	++	+	+	+	+	++	++
SEQ ID NO:7	-	+	+	--	++	+	++	+
SEQ ID NO:1	-+	++	+	--	+	+	++	--

TABLE 7: Heterogeneity in Induction of an Immune Response (IFN-γ)

	1	2	3	4	5	6	7	8
SEQ ID NO:40	-+	++	--	--	--	+	++	+
SEQ ID NO:32	-	++	+	+	++	+	--	--
SEQ ID NO:102	--	--	++	++	++	+	--	++

The foregoing data demonstrates induction of an immune response to an ODN comprising various sequences in human PBMC isolated from individual donors. Specifically, these data demonstrate that a single sequence induces a varied immune response in different donors, as show, e.g., in Table 4, the percent of donors induced varied from 7% to 69%, as measured by IL-6 production, and in Table 5, the percent of donors induced varied from 11% to 93%, as measured by IFN-γ production. Further, as demonstrated in Tables 6 and 7, there was substantial heterogeneity in induction of an immune response in different donors.

Example 3

The following example demonstrates *in vitro* induction of an immune response after administration of multiple ODNs comprising a CpG sequence. Induction of an immune response was measured by production of the cytokines IL-6 and IFN-γ, and cell proliferation in human PBMC isolated from individual donors.

Human PBMC were isolated. as described in Example 1. ODNs were synthesized on a DNA synthesizer (Applied Biosystems Inc., Foster City, CA), as described in Example 1. In some ODNs, the normal DNA backbone

phosphodiesterase linkages were replaced with phosphorothioate linkages, as described in Example 1. To reduce degradation of the ODNs, those that did not have an entire phosphorothioate backbone contained phosphorothioate linkages at the 5' and 3' ends. Cells were incubated for approximately 72 hrs with the various ODNs. IL-6 and IFN-γ levels were determined by ELISA using anti-IL-6 and anti-IFN-γ antibodies, as described in Example 1. Cell proliferation was determined by [^{3}H] thymidine incorporation, as described in Example 1. Results are set forth in Table 8: Induction of an Immune Response to Multiple ODNs.

TABLE 8: Induction of an Immune Response to Multiple ODNs

	IL-6 (ELISA)	IFN-γ (ELISA)	Proliferation ([^{3}H] T)
Donor 1			
SEQ ID NO:1	12.0	2.3	13.0
SEQ ID NO:43	7.0	9.0	1.6
SEQ ID NO:1 + SEQ ID NO:43	27	40.0	19.9
Donor 2			
SEQ ID NO:1	5.0	13.0	37.7
SEQ ID NO:43	2.0	7.0	1.3
SEQ ID NO:1 + SEQ ID NO:43	9.0	33.0	68.6
Donor 3			
SEQ ID NO:1	10.0	8.0	13.1
SEQ ID NO:43	1.0	12.0	1.3
SEQ ID NO:1 + SEQ ID NO:43	8.0	42.0	17.5

The foregoing data demonstrates the induction of an immune response after administration of multiple ODNs comprising various CpG sequences in human PBMC isolated from individual donors. Specifically, these data demonstrate that multiple ODNs synergistically induce an immune response, as demonstrated by, e.g., an increase of 26.6% as measured by cell proliferation, 29.6% as measured by IL-6 levels and 71.7% as measured by IFN-γ levels after administration to Donor 1 of a combination of an ODN comprising SEQ ID NO:1 and an ODN comprising SEQ ID NO:43 compared to the combined immune response of each ODN administered separately.

Example 4

The following example demonstrates *in vitro* induction of an immune response after administration of multiple ODNs comprising a CpG sequence. Induction of an immune response was measured by production of the cytokines IL-6 and IFN-γ in human PBMC isolated from individual donors as compared to unstimulated PBMC from the same donor.

Human PBMC were isolated, as described in Example 1. ODNs were synthesized on a DNA synthesizer (Applied Biosystems Inc., Foster City, CA), as described in Example 1. In some ODNs, the normal DNA backbone phosphodiesterase linkages were replaced with phosphorothioate linkages, as described in Example 1. To reduce degradation of the ODNs, those that did not have an entire phosphorothioate backbone contained phosphorothioate linkages at the 5' and 3' ends. Cells were incubated for approximately 72 hrs with the various ODNs. IL-6 and IFN-γ levels were determined by ELISA using anti-IL-6 and anti-IFN-γ antibodies, as described in Example 1. The percentage of donors induced by the various multiple ODNs by at least 3-fold for IL-6 and 5-fold for IFN-γ are set forth for IL-6 levels in Table 9: Percent Induction of an Immune Response (IL-6) and for IFN-γ levels in Table 10: Induction of an Immune Response (IFN-γ).

TABLE 9: Percent Induction of an Immune Response (IL-6) to Multiple ODNs

	Percent Induction	Number of Donors
SEQ ID NO:7 + SEQ ID NO:1 + SEQ ID NO:108	100%	4
SEQ ID NO:7 + SEQ ID NO:20 + SEQ ID NO:98	100%	2
SEQ ID NO:20 + SEQ ID NO:108 + SEQ ID NO:98	100%	2
SEQ ID NO:7 + SEQ ID NO:20 + SEQ ID NO:1	80%	5
SEQ ID NO:20 + SEQ ID NO:1 + SEQ ID NO:108	80%	5
SEQ ID NO:7 + SEQ ID NO:20 + SEQ ID NO:108	60%	10
SEQ ID NO:7 + SEQ ID NO:108 + SEQ ID NO:98	38%	16

TABLE 10: Percent Induction of an Immune Response (IFN-γ) to Multiple ODNs

	Percent Induction	Number of Donors
SEQ ID NO:43+ SEQ ID NO:40 + SEQ ID NO:106	100%	5
SEQ ID NO:32 + SEQ ID NO:40 + SEQ ID NO:106	100%	5
SEQ ID NO:43 + SEQ ID NO:102 + SEQ ID NO:106	89%	19
SEQ ID NO:32 + SEQ ID NO:40 + SEQ ID NO:42	80%	5
SEQ ID NO:32 + SEQ ID NO:102 + SEQ ID NO:106	40%	5
SEQ ID NO:43 + SEQ ID NO:40 + SEQ ID NO:42	40%	5

The foregoing data demonstrates the induction of an immune response after administration of multiple ODNs comprising various CpG sequences in human PBMC isolated from individual donors. Specifically, these data demonstrate that multiple ODNs synergistically induce an immune response, as demonstrated by, e.g., Table 9, in which the percent induction was increased in two of the multiple ODNs to 100%, as measured by IL-6 production. This is also show in Table 10, in which the percent induction was increased to 100% for three of the multiple ODNs, as measured by IFN-γ production.

Example 5

The following example demonstrates *in vitro* induction of an immune response after administration of a single ODN comprising multiple different CpG sequences. Induction of an immune response was measured by production of the cytokines IL-6 and IFN-γ, and cell proliferation in human PBMC isolated from individual donors.

Human PBMC were isolated, as described in Example 1. ODNs were synthesized on a DNA synthesizer (Applied Biosystems Inc., Foster City, CA), as described in Example 1. In some ODNs, the normal DNA backbone phosphodiesterase linkages were replaced with phosphorothioate linkages, as described in Example 1. To reduce degradation of the ODNs, those that did not have an entire phosphorothioate backbone contained phosphorothioate linkages at the 5' and 3' ends. Cells were incubated for approximately 72 hrs with the various ODNs. IL-6 and IFN-γ levels were determined by ELISA using anti-IL-6 and anti-IFN-γ

antibodies, as described in Example 1. Cell proliferation was determined by [^{3}H] thymidine incorporation, as described in Example 1. Results are set forth in Table 11: Induction of an Immune Response to a Single ODN Comprising Multiple Different CpG Sequences.

TABLE 11: Induction of an Immune Response to a Single ODN Comprising Multiple Different CpG Sequences

	IL-6 (ELISA)	IFN-γ (ELISA)	Proliferation ([^{3}H] T)
Donor 1			
SEQ ID NO:1	5	3	18
SEQ ID NO:43	3	4	3
SEQ ID NO:1 + SEQ ID NO:43	23	7	29
Donor 2			
SEQ ID NO:1	5	3	31
SEQ ID NO:43	2	4	4
SEQ ID NO:1 + SEQ ID NO:43	15	6	57

The foregoing data demonstrates the induction of an immune response after administration of a single ODN comprising multiple different CpG sequences in human PBMC isolated from individual donors. Specifically, these data demonstrate that a single ODN comprising multiple different CpG sequences synergistically induces an immune response, such as is demonstrated by, e.g., an increase in IL-6 levels of 65.2% in Donor 1 and 53.3% in Donor 2 after administration of a single ODN comprising SEQ ID NO:1 and SEQ ID NO:43 compared to the combined immune response of a single ODN comprising either SEQ ID NO:1 or SEQ ID NO:43 when administered separately.

All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and it is intended that the invention may be

practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

WHAT IS CLAIMED IS:

1. A substantially pure or isolated oligodeoxynucleotide (ODN) of at least about 10 nucleotides comprising multiple CpG sequences, wherein at least one of the multiple CpG sequences is different from another of the multiple CpG sequences.

2. The ODN of claim 1, wherein at least one of the multiple CpG sequences is represented by the formula 5' $N_1N_2N_3$T-CpG-W$N_4N_5N_6$ 3', wherein W is A or T, and N_1, N_2, N_3, N_4, N_5, and N_6 are any nucleotides.

3. The ODN of claim 1, wherein at least one of the multiple CpG sequences is represented by the formula 5' RY-CpG-RY 3', wherein R is A or G and Y is C or T.

4. The ODN of claim 3, wherein the sequences on the 5' side of the CpG sequences form a palindrome with the sequences on the 3' side of the CpG sequence.

5. The ODN of any of claims 1-4, wherein at least one of the multiple CpG sequences is selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:112.

6. The ODN of any of claims 1-5, wherein the ODN has a phosphate backbone modification.

7. The ODN of claim 6, wherein the phosphate backbone modification is a phosphorothioate backbone modification.

8. The ODN of any of claims 1-7, wherein the ODN is modified to prevent degradation.

9. The ODN of any of claims 1-8, wherein the ODN comprises about 100 nucleotides or less.

10. The ODN claim 9, wherein the ODN comprises about 50 nucleotides or less.

11. An ODN delivery complex comprising multiple substantially pure or isolated ODNs of at least about 10 nucleotides comprising a CpG sequence and a targeting means, wherein at least one of the CpG sequences is different from another of the multiple CpG sequences.

12. An ODN delivery complex comprising a single ODN or multiple ODNs of any of claims 1-10 and a targeting means.

13. The ODN delivery complex of claim 12, wherein the ODN delivery complex comprises a single ODN of any of claims 1-10.

14. The ODN delivery complex of claim 12, wherein the ODN delivery complex comprises multiple ODNs of any of claims 1-10.

15. The ODN delivery complex of any of claims 12-15, wherein the targeting means is selected from the group consisting of cholesterol, virosome, liposome, lipid, and a target cell specific binding agent.

16. A pharmacological composition comprising multiple substantially pure or isolated ODNs of at least about 10 nucleotides comprising a CpG sequence and a pharmacologically acceptable carrier, wherein at least one of the CpG sequences is different from another of the multiple CpG sequences.

17. A pharmacological composition comprising a single ODN or multiple ODNs of any of claims 1-10 and a pharmacologically acceptable carrier.

18. The pharmacological composition of claim 17, wherein the pharmacological composition comprises a single ODN of any of claims 1-10.

19. The pharmacological composition of claim 17, wherein the pharmacological composition comprises multiple ODNs of any of claims 1-10.

20. A method of inducing an immune response comprising administering to a host multiple substantially pure or isolated ODNs of at least about 10 nucleotides comprising a CpG sequence, wherein at least one of the CpG sequences is different from another of the multiple CpG sequences, to induce an immune response in the host.

21. A method of inducing an immune response comprising administering to a host a single ODN or multiple ODNs of any of claims 1-10 to induce an immune response in the host.

22. The method of claim 21, wherein the method comprises administering to a host a single ODN of any of claims 1-10.

23. The method of claim 21, wherein the method comprises administering to a host multiple ODNs of any of claims 1-10.

24. A method of inducing an immune response comprising administering to a host the ODN delivery complex of any of claims 11-15 to induce an immune response in the host.

25. A method of inducing an immune response comprising administering to a host the pharmacological composition of any of claims 16-19 to induce an immune response in the host.

26. The method of any of claims 20-25, wherein the immune response is a cell-mediated immune response.

27. The method of claim 26, wherein non-B cells are activated in the host.

28. The method of claim 26 or 27, wherein cytokine production in the host is induced.

29. The method of claim 28, wherein the cytokine is IFN-γ.

30. The method of any of claims 20-25, wherein the immune response is a humoral immune response.

31. The method of claim 30, wherein B cells are activated in the host.

32. The method of claim 30 or 31, wherein IL-6 production is induced in the host.

33. The method of any of claims 30-32, wherein antibody production is induced in the host.

34. The method of any of claims 20-33, wherein the induction of an immune response is used to treat, prevent, or ameliorate an allergic reaction, and administration is optionally in combination with an anti-allergenic agent.

35. The method of claim 34, wherein the allergic reaction is asthmatic.

36. The method of any of claims 20-33, wherein the induction of an immune response is used to treat cancer, and administration is optionally in combination with an anti-cancer agent.

37. The method of claim 36, wherein the cancer is a solid tumor cancer.

38. The method of any of claims 20-33, wherein the induction of an immune response is used to improve the efficacy of a vaccine, and administration is optionally in combination with a vaccine.

39. The method of any of claims 20-33, wherein the induction of an immune response is used to treat, prevent or ameliorate a disease associated with the immune system.

40. The method of claim 39, wherein the disease associated with the immune system is an autoimmune disorder.

41. The method of claim 39, wherein the disease associated with the immune system is an immune system deficiency.

42. The method of any of claims 20-33, wherein the induction of an immune response is used in antisense therapy, and administration is optionally in combination with an antisense agent.

43. The method of any of claims 20-33, wherein the induction of an immune response is used to treat, prevent, or ameliorate an infection, and administration is optionally in combination with an anti-infectious agent.

44. The method of any of claims 20-33, wherein the induction of an immune response is used to treat, prevent, or ameliorate the symptoms resulting from exposure to a bio-warfare agent.

45. The method of any of claims 20-44, wherein the host is a human.

SEQUENCE LISTING

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REPRESENTED BY THE SECRETARY, DEPARTMENT
OF HEALTH AND HUMAN SERVICES
KLINMAN, Dennis
ISHII, Ken
VERTHELYI, Daniela

<120> OLIGODEOXYNUCLEOTIDE AND ITS USE TO INDUCE AN IMMUNE RESPONSE

<130> 207509

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<141> 2001-01-12

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INTERNATIONAL SEARCH REPORT

Inte .onal Application No
PCT/US 01/01122

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H21/00 A61K31/70 A61K39/39 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 55495 A (DYNAVAX TECHNOLOGIES CORP ;DINA DINO (US); ROMAN MARK (US); SCHWAR) 10 December 1998 (1998-12-10) tables 1-4 ---	1-45
X	WO 99 58118 A (CPG IMMUNOPHARMACEUTICALS GMBH ;CPG IMMUNOPHARMACEUTICALS INC (US)) 18 November 1999 (1999-11-18) table 1 ---	1-45
X	WO 99 51259 A (UNIV IOWA RES FOUND) 14 October 1999 (1999-10-14) table 1 ---	1-45
X	WO 99 56755 A (OTTAWA CIVIC LOEB RES INST ;UNIV IOWA RES FOUND (US); US NAVY (US)) 11 November 1999 (1999-11-11) table 1 --- -/--	1-45

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 May 2001	28/05/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bardlli, W

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INTERNATIONAL SEARCH REPORT

Inte .onal Application No
PCT/US 01/01122

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 18810 A (UNIV IOWA RES FOUND ;KLINE JOEL N (US); KRIEG ARTHUR M (US)) 7 May 1998 (1998-05-07) tables 10-13	1-45
X	WO 98 40100 A (DAVIS HEATHER L ;OTTAWA CIVIC LOEB RES INST (CA); QIAGEN GMBH (DE)) 17 September 1998 (1998-09-17) page 12	1-45
X	WO 98 49288 A (HYBRIDON INC) 5 November 1998 (1998-11-05) tables 4-8	1-45
X	WO 98 52581 A (WU TONG ;DAVIS HEATHER L (CA); OTTAWA CIVIC HOSPITAL LOEB RES (CA)) 26 November 1998 (1998-11-26) tables 11-14	1-45

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte .onal Application No
PCT/US 01/01122

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9855495 A	10-12-1998	AU 7811398 A	21-12-1998
		AU 7817898 A	21-12-1998
		EP 1003850 A	31-05-2000
		EP 0986572 A	22-03-2000
		US 6225292 B	01-05-2001
		WO 9855609 A	10-12-1998
WO 9958118 A	18-11-1999	AU 4528899 A	29-11-1999
		EP 1078053 A	28-02-2001
WO 9951259 A	14-10-1999	AU 3467899 A	25-10-1999
		EP 1067956 A	17-01-2001
		US 6218371 B	17-04-2001
WO 9956755 A	11-11-1999	AU 3884199 A	23-11-1999
		EP 1077708 A	28-02-2001
WO 9818810 A	07-05-1998	US 6207646 B	27-03-2001
		AU 5242498 A	22-05-1998
		CN 1235609 A	17-11-1999
		EP 0948510 A	13-10-1999
WO 9840100 A	17-09-1998	AU 6759598 A	29-09-1998
		EP 1005368 A	07-06-2000
WO 9849288 A	05-11-1998	AU 7171298 A	24-11-1998
		EP 0991755 A	12-04-2000
WO 9852581 A	26-11-1998	AU 7690898 A	11-12-1998
		EP 1003531 A	31-05-2000